

## Cultural Management of Huanglongbing: Current Status and Ongoing Research

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### ABSTRACT

Huanglongbing (HLB), formerly known as greening, is a bacterial disease restricted to some Asian and African regions until two decades ago. Nowadays, associated bacteria and their vectors have spread to almost all citrus-producing regions, and it is currently considered the most devastating citrus disease. HLB management can be approached in terms of prevention, limiting or avoiding pathogen and associated vectors to reach an area, or in terms of control, trying to reduce the impact of the disease by adopting different cultural strategies depending on infestation/infection levels. In both cases, control of psyllid populations is currently the best way to stop HLB spread. Best cultural actions (CHMAs, TPS system) to attain this goal and, thus, able to limit HLB spread, and ongoing research in this regard is summarized in this review.

**Keywords:** bacterial pathogens, biological control, chemical control, cultural control, disease control and pest management

Being formerly known as likubin in Taiwan, greening in South Africa, mottle leaf in Philippines, dieback in India, phloem necrosis or vein phloem degeneration in Indonesia, or yellow shoot in China, Huanglongbing (HLB) is probably one of the oldest known citrus diseases (Bové 2006). HLB-associated symptomatology has long been confused with that arising from nutritional deficiencies or different abiotic toxicities, until the decade of the 1960s, when the disease was demonstrated to be graft-transmissible (Bové 2006). First symptoms appear as blotchy mottled leaves on individual branches, which slowly spread to other limbs. As infection progresses, affected branches dieback, reducing fruit yield and quality (Bassanezi et al. 2009, 2011).

### HLB DISEASE

It remained as an endemic African and Asian disease until the beginning of 21st century when because of increased trafficking in

goods and people, the disease began to spread rapidly to the rest of the world, with the Mediterranean basin and Australia/New Zealand currently being the only citrus regions that remain free of HLB-associated bacteria (*Candidatus Liberibacter [CLs] asiaticus* [CLAs], *americanus* [CLAm], and *africanus* [CLAf]). The former includes, besides canonical africanus strain, different subspecies, such as *clausenae*, *vepridis*, *capensis*, or *zanthoxyli*, all of them identified in Africa and able to infect rutaceous hosts (Roberts et al. 2017). CLAs is the most widespread bacterial species in Asia and America, and it has almost completely replaced CLAm in Brazil about 4 years after the first report of HLB (Lopes et al. 2009). In the African continent, CLAf is the predominant HLB-associated bacterium, but CLAs has been already reported in Ethiopia (Saponari et al. 2010) and more recently in Kenya (Ajene et al. 2020a).

The spread of HLB-associated CLs is primarily mediated by two psyllid vectors: *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) and *Trioza erytreae* (Del Guercio, 1918) (Hemiptera, Triozidae), native to Asian and African continents, respectively. Infestation of *D. citri* in citrus trees is asymptomatic while *T. erytreae* produces typical globular galls in the underside of infested leaves through nymphs feeding. *T. erytreae* has been already reported in 23 African countries (Table 1), while *D. citri* has expanded throughout Asia reaching Iran and the Arabian Peninsula and has also invaded America, stretching from Argentina to California (U.S.A.) (Table 1). In recent years, it has been also detected in Ethiopia, Tanzania, and Nigeria. Different evidences indicate that both psyllids are able to

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**TABLE 1**  
**Year of first report of Huanglongbing vector (*Diaphorina citri* or *Trioza erytreae*) and year of first report of '*Candidatus Liberibacter asiaticus*' and '*Candidatus Liberibacter africanus*'-associated bacteria for various countries**

Country	<i>D. citri</i>	CLas	<i>T. erytreae</i>	CLaf
Africa				
Ethiopia	2017 <sup>**a,b</sup>	2010 <sup>*b</sup>	1918 <sup>c</sup>	1976 <sup>c</sup>
Angola			2016 <sup>b</sup>	2019 <sup>b</sup>
Cameroon			1967 <sup>c</sup>	1988 <sup>c</sup>
Comoros			1992 <sup>b</sup>	1998 <sup>b</sup>
Democratic Republic of the Congo			1994 <sup>**b</sup>	
Eritrea			1918 <sup>b</sup>	
Eswatini			1969 <sup>**b</sup>	1970 <sup>b</sup>
Gabon			1988 <sup>b</sup>	
Kenya	2015 <sup>**b</sup>	2020 <sup>**b</sup>	1918 <sup>c</sup>	1981 <sup>c</sup>
Madagascar			1961 <sup>c</sup>	1968 <sup>c</sup>
Malawi			1967 <sup>c</sup>	1988 <sup>c</sup>
Mauritius	1973 <sup>b</sup>	1996 <sup>**b</sup>	1973 <sup>b</sup>	1972 <sup>b</sup>
Reunion	1973 <sup>b</sup>	1984 <sup>**b</sup>	1973 <sup>*b</sup>	1980 <sup>b</sup>
Rwanda			1958 <sup>c</sup>	1988 <sup>c</sup>
Saint Helena			1960 <sup>b</sup>	1980 <sup>**b</sup>
Burundi			1958 <sup>c</sup>	1988 <sup>c</sup>
Sao Tome and Principe			1984 <sup>b</sup>	
South Africa			1897 <sup>***c</sup>	1928 <sup>**c</sup>
Sudan			1965 <sup>b</sup>	
Tanzania	2014 <sup>**b</sup>		1967 <sup>c</sup>	1984 <sup>**c</sup>
Uganda			1921 <sup>b</sup>	2015 <sup>b</sup>
Zambia			1965 <sup>b</sup>	
Zimbabwe			1962 <sup>***c</sup>	1981 <sup>**c</sup>
Central African Republic				1995 <sup>b</sup>
Nigeria	2019 <sup>b</sup>			2018 <sup>b</sup>
Somalia				1995 <sup>b</sup>
America				
Argentina	2006 <sup>**b</sup>	2012 <sup>**c</sup>		
Cuba	1998 <sup>c</sup>	2006 <sup>***c</sup>		
Bahamas	2001 <sup>b</sup>			
Barbados	2011 <sup>**b</sup>	2014 <sup>**b</sup>		
Belize	2005 <sup>b</sup>	2009 <sup>**b</sup>		
Amazonas (Brazil)	1968 <sup>b</sup>			
Bahia (Brazil)	1968 <sup>b</sup>	2011 <sup>*b</sup>		
Ceara (Brazil)	1968 <sup>b</sup>			
Para (Brazil)	1968 <sup>b</sup>			
Pernambuco (Brazil)	1968 <sup>b</sup>			

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<sup>a</sup> Infestation/infection levels: \*, low ; \*\*, medium; and \*\*\*, high.

<sup>b</sup> European Plant Protection Office (EPPO).

<sup>c</sup> Bové (2014).

<sup>d</sup> Island territory.

<sup>e</sup> Mainland.

<sup>f</sup> Eradication date.

TABLE 1 (Continued from previous page)

Country	<i>D. citri</i>	CLas	<i>T. erytraeae</i>	CLaf
Rio de Janeiro (Brazil)	1968 <sup>b</sup>			
Santa Catarina (Brazil)	2005 <sup>b</sup>			
Sao Paulo (Brazil)	1942 <sup>c</sup>	2004*** <sup>c</sup>		
Minas Gerais (Brazil)		2009*** <sup>b</sup>		
Parana (Brazil)		2008*** <sup>b</sup>		
Cayman Islands	2004 <sup>b</sup>			
Colombia	2007*** <sup>b</sup>	2015* <sup>b</sup>		
Costa Rica	2005 <sup>b</sup>	2011** <sup>b</sup>		
Dominica	2007 <sup>b</sup>	2012** <sup>b</sup>		
Dominican Republic	2011 <sup>b</sup>	2008** <sup>b</sup>		
El Salvador		2013** <sup>b</sup>		
Guadaloupe <sup>c</sup>	1998*** <sup>a</sup>	2012*** <sup>c</sup>		
Guatemala		2010 <sup>b</sup>		
Haiti	2000 <sup>b</sup>			
Honduras		2010* <sup>b</sup>		
Jamaica	2003 <sup>b</sup>	2009*** <sup>b</sup>		
Martinique	2012 <sup>b</sup>	2013** <sup>b</sup>		
Mexico	2001** <sup>b</sup>	2009** <sup>b</sup>		
Nicaragua		2010 <sup>b</sup>		
Paraguay	2008** <sup>b</sup>	2013** <sup>b</sup>		
Panamá		2016** <sup>b</sup>		
Puerto Rico	2002 <sup>b</sup>	2007*** <sup>b</sup>		
Saint Lucia	2018 <sup>b</sup>			
St Vincent and the Grenadines	2018 <sup>b</sup>			
Trinidad and Tobago		2017** <sup>b</sup>		
Texas (U.S.A.)	2001 <sup>c</sup>	2012*** <sup>c</sup>		
Florida (U.S.A.)	1998*** <sup>c</sup>	2005*** <sup>c</sup>		
Alabama (U.S.A.)	2008* <sup>b</sup>	2017* <sup>b</sup>		
California (U.S.A.)	2008* <sup>b</sup>	2012** <sup>b</sup>		
Georgia (U.S.A.)	2008* <sup>b</sup>	2009*** <sup>b</sup>		
Louisiana (U.S.A.)	2008* <sup>b</sup>	2008** <sup>b</sup>		
Mississippi (U.S.A.)	2008* <sup>b</sup>			
South Carolina (U.S.A.)	2008* <sup>b</sup>	2009** <sup>b</sup>		
Uruguay	1991* <sup>b</sup>			
Antigua y Barbuda	2006 <sup>b</sup>			
Venezuela	1999** <sup>b</sup>	2018** <sup>b</sup>		
Virgin Islands (U.S.)	2010 <sup>b</sup>	2010*** <sup>b</sup>		
Asia				
Japan	1974** <sup>b</sup>	1988** <sup>b</sup>		
Malaysia	1962* <sup>b</sup>	1992** <sup>b</sup>		
Afghanistan	1971 <sup>b</sup>			
Bangladesh	1978 <sup>b</sup>	1978 <sup>b</sup>		
Bhutan	2003 <sup>b</sup>	2003 <sup>b</sup>		

(Continued on next page)

TABLE 1 (Continued from previous page)

Country	<i>D. citri</i>	CLas	<i>T. erytrae</i>	CLaf
Cambodia	1970 <sup>b</sup>	1998 <sup>b</sup>		
Aomen (China)	1927 <sup>b</sup>			
Fujian (China)	1994 <sup>b</sup>	1956 <sup>b</sup>		
Guangdong (China)	1987 <sup>b</sup>	1956 <sup>b</sup>		
Guangxi (China)	1979 <sup>**b</sup>	1956 <sup>b</sup>		
Gizhou (China)	2006 <sup>b</sup>	2014 <sup>b</sup>		
Hainan (China)	2006 <sup>b</sup>	1956 <sup>b</sup>		
Henan (China)	1935 <sup>b</sup>	2009 <sup>b</sup>		
Hunan (China)	2006 <sup>b</sup>	2009 <sup>b</sup>		
Jiangxi (China)	2006 <sup>b</sup>	1956 <sup>b</sup>		
Sichuan (China)	2006 <sup>b</sup>	2009 <sup>b</sup>		
Xianggang (China)	1970 <sup>***b</sup>	1992 <sup>b</sup>		
Yunnan (China)	2006 <sup>b</sup>	2011 <sup>b</sup>		
Zhejiang (China)	1989 <sup>b</sup>	1956 <sup>b</sup>		
East Timor	2004 <sup>b</sup>	2000 <sup>b</sup>		
India	1959 <sup>b</sup>	1960 <sup>b</sup>		
Indonesia	1927 <sup>b</sup>	1984 <sup>b</sup>		
Iran	2006 <sup>**b</sup>	2008 <sup>**b</sup>		
Laos	2000 <sup>b</sup>	1998 <sup>b</sup>		
Maldives	1990 <sup>b</sup>			
Myanmar	1993 <sup>b</sup>	1998 <sup>b</sup>		
Nepal	1972 <sup>b</sup>	1972 <sup>***b</sup>		
Oman	2005 <sup>**b</sup>	2018 <sup>**b</sup>		
Pakistan	1988 <sup>b</sup>	1988 <sup>b</sup>		
Philippines	1970 <sup>b</sup>	1950 <sup>***b</sup>		
Saudi Arabia	1972 <sup>b</sup>	1984 <sup>b</sup>	1974 <sup>**b</sup>	1984 <sup>**b</sup>
Singapore	1993 <sup>**b</sup>			
Sri Lanka	1970 <sup>b</sup>	1996 <sup>b</sup>		
Taiwan	1907 <sup>**b</sup>	1951 <sup>***b</sup>		
United Arab Emirates	2008 <sup>b</sup>			
Vietnam	1978 <sup>**b</sup>	1992 <sup>**b</sup>		
Yemen	1938 <sup>b</sup>	1986 <sup>**b</sup>	1986 <sup>**b</sup>	1984 <sup>**b</sup>
Thailand	1993 <sup>b</sup>	1960 <sup>b</sup>		
Europe				
Portugal			1994 <sup>**b,d</sup> /2015 <sup>**b,e</sup>	
Spain			2002 <sup>**b,d</sup> /2014 <sup>**b,e</sup>	
Oceania				
American Samoa	2011 <sup>b</sup>			
Australia	1915/1922 <sup>b,f</sup>			
Northern Territory	1915/1922 <sup>b,f</sup>			
Guam	2008 <sup>b</sup>			
Northern Mariana Islands	2011 <sup>b</sup>			
Papua New Guinea	2002 <sup>**b</sup>	2002 <sup>**b</sup>		

transmit either CLAs or CLaf (Ajene et al. 2020a), but the higher heat tolerance of CLAs and its native vector *D. citri* along with climate warming makes this combination of bacteria/vector the one with the most potential to spread according to different predictive models. Furthermore, although severe symptoms associated to CLaf have been recently reported in Uganda (Ajene et al. 2020a) and Kenya (Richard et al. 2018), CLAs spreads more rapidly, is more difficult to control, and induces more severe HLB symptoms in commercial citrus orchards and, thus, higher economic impact. The presence of *D. citri* and CLAs in Central and South Africa accentuates the threat to the citrus industry of this continent, currently already affected by the least severe HLB form associated to CLaf and *T. erythrae* (Ajene et al. 2020a). Also, the European Union is under high risk because, despite both psyllids being cataloged as quarantine pests years ago, *T. erythrae* is by this time expanding along north and west of the Iberian Peninsula, getting closer to important citrus regions from Spain and Portugal (Benhadi-Marín et al. 2021; Vasco et al. 2020). HLB-associated CLs and *D. citri* have not been yet found in the European Union, but the risk of entrance is extremely high. For example, several interceptions of *D. citri* in legal plant imports have been reported despite the phytosanitary restrictions they are subjected to, while illegal imports constitute another high-risk pathway of potential entrance (Bragard et al. 2020).

In 2006 Prof. Bové indicated that, once HLB vectors are present, CLs are not very far (Bové 2006). In the last 15 years, the registration dates of new infections/infestations by CLs/psyllids not only confirms this observation but also indicates that the time lapse between vector and bacterial detection is decreasing (Table 1). Predictive mathematical models indicate high aptitude for *D. citri* and CLAs to settle in citrus areas from Italy, Greece, Croatia, Corsica, Malta, Cyprus, and some small areas of Southern France (Bragard et al. 2020; Gutierrez and Ponti 2013; Narouei-Khandan et al. 2016; Shimwela et al. 2016; Taylor et al. 2019). The coast of East Africa, part of South and Central Africa, and some areas in West Africa, in which temperature regimes are limiting *T. erythrae* and CLaf spread, are highly suitable for *D. citri* and CLAs establishment (Shimwela et al. 2016). In all these threatened regions, citrus farms are predominantly small and, thus, more exposed to primary infections from neighboring areas (Bassanezi et al. 2013). The high HLB incidence that can be reached in all these regions if *D. citri* and CLAs are expanded would most probably destroy their citriculture. Thus, efforts from farmers, related stakeholders, and phytosanitary authorities are required to prevent introduction and further spread of these organisms (Ajene et al. 2020a; Shimwela et al. 2016). Northeast Australia is also a hotspot, because of its climate and because HLB is widely spread in the Asia-Pacific region, although it is still free of the disease because of the effectiveness of their bio-security measures (Narouei-Khandan et al. 2016). Potential spread of CLaf in South and Central America, Asia, and Australia as well as marginal suitability in certain European regions has been reported, and potential suitable habitats increase if climate warming is considered (Ajene et al. 2020b). In Kenya, *T. erythrae* adequate habitats embrace large areas of the west and central country and some coastal regions (Richard et al. 2018), while its recent introduction in the Iberian Peninsula (Pérez-Otero et al. 2015) is predicted to spread along north and west coastal regions (Benhadi-Marín et al. 2020).

## A PIECE OF HISTORY ABOUT HLB MANAGEMENT

Since years 2004 and 2005, when CLAs was first detected in two of the most commercially relevant worldwide citricultures, namely those of Sao Paulo (Brazil) and Florida (U.S.A.) States, research on HLB became a priority (Halbert 2005; Teixeira et al. 2005). In both cases, *D. citri* was already present but unmanaged, as in absence of HLB-associated CLs, it is a nonrelevant citrus pest. *D. citri* was first detected in Florida in 1998, and in two years it was spread over the southern half of the peninsula (Halbert et al. 2000). The huge psyllid populations joined to its high efficiency for CLAs transmission led to

a quick bacterial dispersion within few years after its first detection, which has caused billions of dollars of losses to both citrus industries (Bassanezi et al. 2020; Singermann and Useche 2016). In Florida, where CLAs infected trees are maintained to maximize their economic profitability, HLB infection has increased production costs by 283% (Singerman et al. 2018) while production has decreased by 74% (Fried and Hudson 2019) and the number of citrus growers, juice processing facilities, and packinghouses have downsized more than 60% (Singerman et al. 2018), entailing multibillion losses in revenues and the loss of thousands of jobs (Hodges and Spreen 2012). The citrus industry in Florida now faces the problem of establishing new groves to recover an economically profitable citrus industry, a real challenge in a severe HLB-infected/*D. citri*-infested region (Singerman et al. 2018). In South China, where HLB affects citrus regions from the beginning of the 20th century (or earlier, being unidentified until then), working with healthy plant material for new plantations and applying insecticide treatments often but without removing HLB-infected trees also led to quick HLB spread (Zheng et al. 2018). Experience from these two countries indicates that HLB control without removing infected trees is totally unsuccessful. In Sao Paulo State, as in Florida, *D. citri* was widespread when first CLAs and CLam infection was detected. Following phytopathologists recommendations (Bové 2006; Lin 1963), a three-pronged system (TPS) was quickly implemented to stop HLB spread (Belasque et al. 2010). It consists of (i) planting of certified healthy material; (ii) removal of inoculum sources-infected trees; and (iii) application of insecticide treatments to control psyllid populations. Even with this, the number of citrus farms has declined since 2004 by about 34% but production has increased 1.7-fold (Bassanezi et al. 2020). Increase in HLB incidence was doubled in initial years of the epidemics, but from 2012 on, by continuously improving TPS, its occurrence increased by less than 1.5% (Fundecitrus 2019).

Multibillion dollars have been invested worldwide in research to find a cure to HLB, but no efficient, economic, and environmental sustainable one has been found so far, although multiple lines of investigation with potential to develop an environmentally and economically sustainable solution to HLB are still ongoing (Alquézar et al. 2021a; Andrade et al. 2020; NASC 2018; Zuñiga et al. 2020). For example, resistance traits to CLAs infection, which could be introgressed into commercial cultivars, have been found on citrus-related germplasm from Oceania (Alves et al. 2020). However, generation of HLB-resistant citrus cultivars, by sexual crosses or biotechnological strategies, will still take years. Meanwhile, the only systems that have been proven to be efficient to deal with HLB are those based on conventional management, which are the object of this review. To address it, the review is divided in two sections. First, a brief update of the latest developments on management techniques to control *D. citri*/*T. erythrae* populations, which have been proven efficient for field assays, is presented (Fig. 1). In laboratory settings, promising results have been obtained from many different experimental approaches, but they are not included in this work. Second, how to apply the different management strategies depending on psyllid infestation level and the presence/absence of HLB-associated CLs is also reviewed here.

## HLB-PSYLLIDS INFESTATION MANAGEMENT

**Areawide management programs.** Broad spectrum insecticides from various chemical classes (pyrethroids, organophosphates, neonicotinoids, carbamates, and diamides) have been commonly applied to reduce *D. citri* populations (Bassanezi et al. 2020; Boina and Bloomquist 2015; Li et al. 2020; Miranda and Ayres 2020; Miranda et al. 2021b). Organophosphates and pyrethroids are also used to control *T. erythrae* (Rizza et al. 2020). However, the numerous treatments (more than 12) applied year-round in Asian HLB-affected regions are costly, negatively affect the environment, and they are also resulting in psyllid resistance to several insecticides (Hu et al. 2020; Tian et al. 2018). Moreover, insecticide treatments are

effective for reducing *D. citri* population within an orchard, reducing secondary infections, but they are ineffective for limiting HLB primary infections (Bassanezi et al. 2013; Bergamin Filho et al. 2016). Depending on the external inoculum pressure, the primary infections, namely those incited by CLAs transmission by infective psyllids raised in infected trees outside the commercial citrus orchards, cannot be completely avoided even with insecticide applications. It happens because of the failure of insecticide applications to completely cover new shoots and the low residual period of control caused by shoot growth, rain-washing of products, and/or product degradation that creates opportunities for psyllids to feed in the shoots and consequently transmit the pathogen (Bassanezi et al. 2020; De Carli et al. 2018). It was demonstrated that citrus growers should extend the disease management measures to areas around their orchards (radius  $\approx$  5 km) to achieve successful HLB control (Bassanezi et al. 2013, 2020; Miranda and Ayres 2020). Thus, implementation of areawide management programs is essential to avoid primary infections, responsible for HLB spread (Bassanezi et al. 2013, 2020; Yuan et al. 2021; Zheng et al. 2018). Execution of areawide management represents less than 10% of total cost of HLB control adopted within the farm and reduces the infection rate up to 75% (Bassanezi et al. 2020). This kind of program, directed to control vector populations and HLB infections simultaneously at a regional scale, have been already launched in Sao Paulo, Minas Gerais, and Paraná States (Brazil), Florida, California and Texas (U.S.A.), Mexico, and Argentina among other regions. The programs are based on coordinating spray efforts among neighboring areas, carrying them out at those times when a real-time alert system indicates that psyllid populations are increasing in the area (Bassanezi et al. 2020; Graham et al. 2020). *D. citri* individual counts are performed biweekly by farmers by visual inspections or by using yellow sticky traps located in trees at the border of their properties, and automatically updated using a web application coordinated by a central institution (such as University of Florida [U.S.A.], the California Department of Food and Agriculture [CDFA], or Fundecitrus in Brazil). These institutions are also responsible for crews' technical training to unequivocally identify *D. citri* individuals and plants showing early-HLB symptoms. Periodic inspections in the orchards to detect HLB-infected symptomatic trees is complementary but essential, because applying other control measures without removing CLAs inoculum reservoirs in the presence of *D. citri* inevitably leads to HLB spread, as demonstrated by the experience of Florida and Texas, with incidence raises from 0.2% in 2006 to 80% in 2013 and from <0.1 in 2012 to 38.7% in 2017, respectively (Graham et al. 2020; Sétamou et al. 2020). In the Chaoshan region of China, new citrus plantations were devastated in less than 15 years (Zheng et al. 2018). The regional management includes psyllid control and eradication of symptomatic trees into a commercial citrus orchard and in neighboring noncommercial citrus areas, such as abandoned groves,

green spaces, or backyard trees (Bassanezi et al. 2020; Miranda and Ayres 2020). In this regard, the release of the psyllid parasitoid, *Tamarixia radiata* Waterston, 1922 (Hymenoptera: Eulophidae) has been proven useful to reduce psyllids populations in noncommercial areas (Diniz et al. 2020).

**Optimization of insecticide treatments.** In order to minimize economic and environmental impacts, the optimal spray volume and timing for insecticide treatments has been investigated. New flushes are the preferred developmental stage for *D. citri* to feed and oviposit, and, thus, peak populations occur coincident with leaf flushes (Cifuentes-Arenas et al. 2018). Also, at this leaf developmental stage, *D. citri* presents maximum CLAs acquisition/inoculation efficiency (Hall et al. 2016; Lopes and Cifuentes-Arenas 2021; Sétamou et al. 2016). Maximum target population and the short residual period of insecticides on young flushes requires repeated application to leaf flushes during these periods (De Carli et al. 2018). High frequency of insecticide treatments is also recommended for orchards with young, freshly pruned or irrigated trees, because of their higher sprouting at the same time (Cifuentes-Arenas et al. 2018). In young citrus orchards (<3 years old), it is also recommended to apply systemic (neonicotinoid) insecticides as a soil drench and/or trunk associated with insecticide foliar sprays (Miranda and Ayres 2020). It has been recently shown that drench application of systemic insecticides reduces psyllid phloem ingestion and, thus, their ability to inoculate CLAs to healthy plants by 44 (imidacloprid) to 88% (thiamethoxam) (Carmo-Sousa et al. 2020). Because the egg to adult psyllid life takes a minimum of 14 days (Nava et al. 2007), weekly treatments are recommended in flushing periods (Bassanezi et al. 2020). Similarly, in order to reduce the amounts of active ingredients required, dosage optimization studies are ongoing. For *D. citri* control, reduction of the volume for spraying and the insecticide per application was reported to be up to 64% (Bassanezi et al. 2020). It is also recommended to intensify insecticide spraying in the first 100 to 200 m of orchard borders, where psyllids prefer to settle and then the primary HLB infection mostly occurs (Bassanezi et al. 2013; Sétamou and Bartels 2015).

**Cultural techniques.** Incoming psyllids from neighboring areas tend to colonize first perimeter trees where they may spend a whole generation before moving into the orchard (Bassanezi et al. 2013; Sétamou and Bartels 2015). This preference for edge trees can be exploited in different ways to optimize psyllid management. For example, installing height psyllid-resistant fences 6.1 m away from border trees can reduce psyllid population within a grove up to 98% (Sétamou et al. 2018). The presence of tall trees (e.g., *Corymbia torelliana* and *Pinus palestris*) as windbreaks also reduce *D. citri* populations in orchard perimeters (Martini et al. 2015). Whether this reduction is a consequence of being a physical barrier or if it is related to alterations in microclimate is still to be determined. In any case



**FIGURE 1**

Representative photographs of cultural strategies used to control Huanglongbing (HLB) spread. **A**, Example of kaolin-treated plants. **B**, Example of border trap-crop. *Murraya paniculata* plants (front tree, marked with an arrow) used as psyllid trap fence to limit primary infections in the first row/s of orange trees in a commercial grove. Yellow sticky traps to monitor *Diaphorina citri* population are located at different points of the orchard (marked with stars). **C**, Yellow sticky trap, located on the upper side of an adult citrus tree, to monitor *D. citri* population.

their effect could be improved by planting *D. citri* high-attractant plants, ideally resistant to CLAs, which could be used as trap-and-kill crops. For example, growing insecticide-treated orange jasmine in the border of new citrus orchard reduces the number of psyllids within the orchard up to 83% and HLB incidence up to 43% (Tomaseto et al. 2019). Enclosing citrus trees, from planting to maturity, under protective psyllid-resistant screen (CUPS) avoids HLB infection, but the economic viability of this solution is still under study (Ferrarezi et al. 2019).

Interfering with visual cues used by psyllids to find their hosts can be useful to limit HLB spread. For example, using metalized mulch repels *D. citri* and delays HLB infection (Croxtton and Stansly 2014). Alternatively, spraying citrus trees with processed kaolin reduces psyllid infestation by 69% (Hall et al. 2007) and by 80 to 90% if applied preventively when trees are flushing (Miranda et al. 2021a). The whitish aspect of the trees and increased light reflectance in leaves are probably the main mechanisms by which processed kaolin interferes on *D. citri* host selection. Red-kaolin treatment (Pierre et al. 2021) and ultraviolet (UV)-blocking barriers (Miranda et al. 2015) also interfere with *D. citri* host finding ability and limit their dispersion.

Attract-and-kill devices, mostly based on the well-known yellow sticky traps used to monitor *D. citri* population, are also being optimized to control this pest. For example, the addition of magnesium oxide to the traps increases their UV reflectance and, consequently, psyllid attraction (George et al. 2020b). Because magnesium oxide also increases the probing behavior of the psyllid, its combined use with insecticide release devices may increase the number of killed individuals (George et al. 2020b). These kind of devices can be further improved, for example, by incorporating olfactory cues to attract even more psyllids. For example, traps baited with a putative sex pheromone captured more psyllids than no baited ones (Zanardi et al. 2018). Recently, it has been reported that cylindrical yellow traps impregnated with an attractive lure and a pyrethroid were more effective in attracting and killing psyllids than standard yellow sticky traps (George et al. 2020a).

**Ecofriendly HLB management.** Some entomopathogenic fungi (EPF), such as *Beauveria bassiana*, *Cordyceps fumosorosea*, and *Hirsutella citriformis*, have been reported as good killers of *D. citri* in controlled experiments, producing mortality rates close to 100% of adult individuals (Corallo et al. 2021; Du et al. 2020; Pérez-González et al. 2016; Saldarriaga Ausique et al. 2017). To be used in the field, the efficiency of each EPF to control *D. citri*/*T. erytrae* and the best way to deliver it should be tested on each region, as their viability and persistence is highly influenced by environmental conditions. In Sao Paulo State conditions, field studies have demonstrated that *C. fumosorosea* (ESALQ-1296 strain) was highly effective in controlling adults of *D. citri* (mortality > 80%) (Saldarriaga Ausique et al. 2017). Based on these results, a commercial biopesticide (Challenger, Koppert) was released on 2018 as a sustainable tool to manage *D. citri*. Currently, commercial formulations of several EPF are being evaluated against *D. citri* and *T. erytrae*. For example, in Colombia, commercial formulations of *B. bassiana* are able to reduce field *D. citri* populations between 33 and 62%, being most effective at the nymphal stage of the insect (Ramírez-Godoy et al. 2018), probably because of their reduced movement.

Different parasitoids have been reported to be useful to control the HLB-associated psyllids populations. *T. radiata* is the most used/tested one against *D. citri* probably because in Reunion Island, rearing and massive release in 1978 was key to almost eradicating psyllids (Étienne et al. 2001). Just 1 year after first *D. citri* detection (1998), a similar eradication plan based on *T. radiata* release was initiated in Guadeloupe, achieving a good level of control at least temporarily (Étienne et al. 2001). However, nowadays high *D. citri* populations combined with the presence of CLAs in both islands are decimating the citricultures of Reunion and Guadeloupe (Reynaud

and Morillon, personal communications). *T. radiata* was introduced in different citrus growing regions to control *D. citri*, but its impact was variable depending on abiotic (especially temperature) and biotic factors (Li et al. 2018). Establishment of the parasitoid does not control sufficiently the populations of *D. citri* in commercial orchards in the United States, Brazil, or in Asia (India, Taiwan, Vietnam). For example, in Florida (U.S.A.), *T. radiata* contributed less than 1.5% to psyllids mortality and this lack of efficiency has been related with the possible presence of *T. radiata* parasitoids (Michaud 2004), and in Texas (U.S.A.), despite multiple releases, do not control *D. citri* in unmanaged residential trees (Sétamou et al. 2018). However, in California (U.S.A.), Puerto Rico, and Sao Paulo (Brazil), massive releases of parasitoids have led to high parasitism rates (60 to 74%), and it is likely limiting *D. citri* populations in urban areas and abandoned citrus orchards (Marin 2019; Milosavljevi et al. 2021; Pluke et al. 2008), although not eradicating it. *T. erytrae* parasitoids from South Africa and Zimbabwe were first described in 1963 and 1972 (Van den Berg and Fletcher 1988), but published scientific works regarding their efficacy in reducing psyllids populations are still scarce. The most tested parasitoid against *T. erytrae* is *Tamarixia dryi* (Waterson), which almost eradicated the psyllid from Reunion and Mauritius (Van Den Berg and Greenland 2000). As for *T. radiata*, establishment of *T. dryi* in a specific region is determined by biotic and abiotic factors and should be evaluated for each case (Pérez-Rodríguez et al. 2019). In Canary Islands (Spain), *T. dryi* was released in spring 2018, and the first data indicate that it is effectively reducing the spread of *T. erytrae*, although it has not been able to eradicate it (Tena 2020). Eradication of the HLB psyllid vectors by *Tamarixia* species does not occur because it would mean consequent eradication of *T. radiata* and *T. dryi* in the area as *D. citri* and *T. erytrae* are usually their only hosts for feeding. Besides *Tamarixia* spp., some other psyllid parasitoids, such as *Diaphorencyrtus aligarhensis* (Shafee, Alam, and Agarwal), native from India and also reported in Philippines and China, and the South African *Psyllaephagus pulvinatus* (Waterston), have been reported (McDaniel and Moran, 1972; Rohrig et al. 2011). However, to our knowledge, attempts to establish these parasitoids in other regions have failed. The use of generalist predators (lacewings, lady beetles, or mites) to control HLB-associated psyllids is also being tested (Kalile et al. 2021; Khan et al. 2016, 2020). The ability of several polyphagous insects to manage *T. erytrae* is envisaged from field observations (Molina et al. 2021). However, field efficacy tests of these alternative biological control agents are still pending.

Push-pull strategies, which have been successfully applied in other crops, could be also used to limit HLB spread (Yan et al. 2015). They are based in the use of a repellent or host-masking stimuli (push) joined with attractant lures away from the host (pull). Push/pull components include nontoxic visual and/or olfactory cues that should also be tested in field conditions to study their real efficiency. In Vietnam, several years ago it was reported that in guava-citrus intercropped orchards *D. citri* populations were low and, thus, HLB infection was delayed (Beattie et al. 2006), and that this effect was related to deterrent volatile emitted from guava leaves (Rouseff et al. 2008). These results indicate that a push/pull strategy may be feasible to control HLB-associated vector populations, but from an economic perspective, it should be optimized to lose less yield because of intercropping, especially in big farms. Investigations on deterrent volatiles/plants is still ongoing (Yan et al. 2020). A transgenic nonattractive orange cultivar is being tested in the field (Alquézar et al. 2021b) combined with the use of a trap border crop. The repellent effect of volatiles emitted from garlic and guava plants and their potential use to pull *T. erytrae* has been also recently reported (Antwi-Agyakwa et al. 2021). Investigations to find optimum pull plants is active as well (Beloti et al. 2017; Tomaseto et al. 2016). Attractant lures, as for example those based on pheromones or host volatile cues, are also being investigated to increase captures in

trapping devices, which may also contribute to the pull side of the strategy (Martini et al. 2020; Zanardi et al. 2019).

Some mineral and essential oils induce a significant reduction on *D. citri* nymphs or/and adult populations in field assays (Orozco-Santos et al. 2016; Rizvi et al. 2018; Tansey et al. 2015), although their application did not slow HLB spread in Malaysia and Florida (Hall et al. 2013; Leong et al. 2012). Field effectiveness of other phytochemicals is also poorly documented. Extracts of garlic and chili pepper (Ramírez-Godoy et al. 2018) and some alkaloids, such as tropane and matrine (Khan et al. 2014; Zanardi et al. 2015), somewhat reduced *D. citri* field populations. It is important to remark that, although laboratory results are promising, up to date, none of these ecofriendly strategies induces mortality rates high enough to effectively control the insect vector (Khan et al. 2014) and so they should be used in combination with other management tools (Kuhns et al. 2016), in cases of high infestation levels, or only in organic orchards if psyllid populations are low and CLs are not present in the infested areas. Other new promising strategies to control *D. citri*, such as BT toxins or antimicrobial peptides (Dorta et al. 2020; Fernandez-Luna et al. 2019; Huang et al. 2021), are currently under development and their performance in the field is still to be evaluated. Nutritional treatments to reduce the impact of HLB in tree yield are being tested in the field, but none of them has been proved consistently effective (Bassanezi et al. 2021; Gottwald et al. 2012; Li et al. 2020; NASC 2018; Stansly et al. 2014; Vashisth et al. 2019; Zuñiga et al. 2020).

**Pest-risk analysis tools and suitability models.** It is essential to identify potential entrance points for psyllids and CLs to avoid them. One of the still-frequent entry ways is via the legal import of plant material. For example, in the last decade, 21 records of *D. citri* interception have been recorded by Europhyt (European Union Notification System for Plant Health Interceptions) (Bragard et al. 2020). Illegal imports of *Citrus* spp. and other citrus relative hosts may also bring HLB-associated pathogens to otherwise HLB-free areas (Bragard et al. 2020). For example, introduction of *D. citri* via infested plant material in Nigeria may have been avoided with stronger quarantine measures (Oke et al. 2020). Strong vigilance at identified potential entry points is the best way to prevent pest/disease spread. For example, in Mexico, Veracruz has been identified as a putative hotspot for introduction and establishment of *T. erythrae*, which can contribute to extent CLas infection to habitats not suitable to *D. citri* (Espinosa-Zaragoza et al. 2021). To accomplish this goal, it is essential to perform pest risk analysis (PRA) that will allow identification of most favorable areas for CLs/psyllids entry and the involvement of phytosanitary authorities. For example, Australia (<https://www.agriculture.gov.au/biosecurity/risk-analysis/memos/2011/baa-201124-final-pest-risk-analysis>) and the European Union (Bragard et al. 2020) have already elaborated PRAs and legislation for HLB-related organisms. Another important point is to detect the introduction of CLs/psyllids as soon as possible. In preventive programs, vigilance is mostly based on visual inspection of characteristic leaf asymmetric mottling to detect bacterial infections and surveys to evaluate vectors presence and symptoms. Experience from countries/regions already dealing with HLB indicates that raising society awareness of the devastating economic and social impacts of this disease, with special attention to farmers and stakeholders, improves the surveillance system and limits the illegal movement of material that may be a gateway entrance to the disease and/or its vectors. Some advertising campaigns to increase society HLB awareness are shown in Figure 2.

Based on climatological conditions and the presence of potential insect vector and bacterial hosts, mathematical models can be used to predict how and where psyllids and CLs would spread if they were introduced. These suitability models also include many other factors, such as movement of goods and people, the ongoing management programs, phenological calendar of citrus varieties, or the amount of psyllids/CLs if they are already present. These models identify in which regions psyllids are most prone to establish/disperse, allowing

to increase preventive control measures in these high-risk areas (Taylor et al. 2019). In addition, once vector/disease is present, this predictive tool allows more efficient psyllid monitoring and control and it even influences establishing suitable legislation aimed to control disease spread (García-Figuera et al. 2021; McRoberts et al. 2019). Maximum optimization of HLB control is achieved when epidemiological models are combined with data for psyllid spread and abundance, CLs detection, and plant flush onset, thus providing an excellent PRA Tool. All these data are collected from farmers via web or app services and updated regularly. Skilled real-time analysis of information coming from wide areas (multiple farmers/orchards) leads to early detection of psyllid population increases or new focus of CLs inoculum to be aware of, allowing rapid intervention management measures, such as insecticide coordinated sprays. These kinds of tools are usually managed by public authorities/institutions (Table 2) and farmers' participation is voluntary. Moreover, a list of potential Rutaceae hosts alternative to commercial citrus is provided by regulatory worldwide authorities, but confident studies on *T. erythrae* and *D. citri* host ranges within Rutaceae are still scarce, incomplete, or unreliable.

## HLB CONTROL IN DIFFERENT SCENARIOS

**High psyllid infestation and widespread presence of HLB-associated bacterium.** Experience from regions under high *D. citri* infestation and CLas infections, such as Sao Paulo State (Brazil), Florida (U.S.A.), and Guangzhou region (China), provides best management practices under this scenario, which have been recently reviewed in detail (Bassanezi et al. 2020; Graham et al. 2020; Li et al. 2020; NASC 2018). In brief, nurseries should be under protective screen to ensure the production of healthy material for replanting, which should be certified before its use. It also should be mandatory to early detect and to quickly remove any source of CLas inoculum to efficiently stop HLB spread. This measure joined to psyllid population control is the only economically sustainable strategy to fight HLB in the long term (Singerman and Rogers 2020; Zhang et al. 2021). In addition, insecticidal treatments should be applied within regional management programs, which take into account vector infestation level and tree phenological stages. For example, weekly insecticide applications are recommended in the flushing periods (De Carli et al. 2018). The synchrony of insecticide treatments along wide areas increases their efficiency and reduces greatly the chances of primary infections (those coming from neighboring lands) to occur (Bassanezi et al. 2013; Bergamin Filho et al. 2016; Yuan et al. 2021). In Mexico, where *D. citri* is spreading since 2001, a similar phytosanitary plan was implemented the same year (2009) that CLas was first detected, remaining HLB-free, five years later, 10 of the 23 citrus production States (Ramírez et al. 2016). In brief, in these scenarios the best way to fight HLB infection is to quickly eliminate CLas sources and to reduce as much as possible *D. citri* amount using, in a coordinated manner, aggressive chemical treatments as required by fluctuating vector population levels and taking into account control of psyllids and inoculum sources outside the commercial orchards, in neighbor surroundings (Bassanezi et al. 2020; Graham et al. 2020; Li et al. 2020).

For *T. erythrae*, the same basic management scheme should be applicable, although considering CLs infection or not as well as specific vector biology, spread, and host range characteristics, which are still to be defined properly, at least in Europe. To our knowledge, areawide control management is not being applied in any African country to control CLs and their vectors.

**Medium psyllid infestation and reduced presence of HLB-associated bacteria.** In newly infected citrus-producing areas such as California (U.S.A.), in the frame of an areawide pest management program, eradication to remove sources of inoculum is being implemented to prevent HLB from becoming endemic. The program implemented by the CDFA includes a statewide early



detection program and coordinated application of chemicals to reduce *D. citri* populations (Li et al. 2020). If a tree tests positive for CLAs, a quarantine surrounding area of 400 m is defined and every tree within the area is inspected for CLs by qPCR (Graham et al. 2020). To control psyllids on backyards and other

noncommercial citrus areas, *T. radiata* is routinely released (Li et al. 2020). The CDFA has also established quarantine measures to restrict plant movement from *D. citri* infested areas and from nurseries to retail outlets, where citrus stocks should be screen-protected (Byrne et al. 2018). Nursery plants are grown under



**FIGURE 2**

Action plans to aware and prevent Huanglongbing (HLB) in different countries. **A**, Citrus Insider, funded by California citrus growers and administered by the California Department of Food and Agriculture, is managed by the Citrus Pest & Disease Prevention Program Committee, which established the statewide work plan and advises the California Secretary of Agriculture and the agricultural industry about efforts to combat serious pests and diseases that could threaten California's citrus industry, been focused in HLB prevention (<https://citrusinsider.org/psyllid-and-disease-control/>). **B**, Gardener's Path provides guides and troubleshooting tips for dealing with pests and plant diseases (<https://gardenerspath.com>) focusing on HLB in this issue. **C**, The Texas Citrus Pest and Disease Management Corporation is focused in the management and control of pests and diseases, including the Asian citrus psyllid and citrus greening, by planning, carrying out, and operating a suppression program while incorporating an areawide integrated pest management approach (<https://www.citrusalert.com/es/>). **D**, The Citrus Greening Guide by Texas A&M AgriLife Extension Service is an education agency with a statewide network of professional educators and county offices with collaborative programs that provide resources, teaching, and extension services for a diverse assortment of topics, among them HLB disease prevention (<https://agrilifeextension.tamu.edu/library/gardening/citrus-greening-guide/>). **E**, The Regional Committee of Plant Health of the Southern Cone (COSAVE) is a plant protection committee constituted by Argentina, Bolivia, Brazil, Chile, Paraguay, Peru, and Uruguay to collaborate in the area of plant protection, in the development of a regional strategy to monitor, and control the transboundary plant pests (<http://www.cosave.org/pagina/bienvenidos-al-comite-de-sanidad-vegetal-cosave>). **F**, The Plant Health and Pest Prevention Services Division from the California Department of Food and Agriculture is focused in scientific assessments of plant pests and diseases and provide expert information of plant pest, maintaining a high degree of awareness regarding emerging plant pest distribution, impacts, and control method (<https://www.cdfa.ca.gov/plant/>). **G**, California Ag Today is an essential source for coverage of California's agriculture news and on plant disease prevention (<https://californiaagtoday.com/>). **H and I**, Informative infographic on HLB from the National Service of Health and Agri-food Quality (SENASA) of the Argentine government (<https://www.argentina.gob.ar/senasa>). **J**, PRE-HLB consortium is developing and implementing a contingency plan to protect the citrus sector in the European Union from HLB disease drivers and cocreating new solutions to manage the disease through a multi-disciplinary approach and in collaboration with experienced partners from America and Asia (<https://www.prehlb.eu/>). **K**, Citrus greening prevention campaign from Save our Citrus (<http://saveourcitrus.org/>). **L**, Fund for Citrus Protection (Fundecitrus), an association maintained by citrus growers and juice manufacturers from the State of São Paulo, foster the sustainable development of the citrus industry, focusing in recent years in the research, protection and prevention of HLB (<https://www.fundecitrus.com.br/>). **M**, Symrise company blog information about citrus greening disease (<https://citrus.symrise.com/a-sustainable-source/#citrus-greening-disease->). **N**, News from The Himalayan Times to raise awareness about HLB disease (<https://thehimalayantimes.com/>).

psyllid-exclusion screen to ensure the production of healthy plant material. By applying this management program in California, CLAs infection is currently limited to backyards and has not been detected in any commercial citrus orchard yet (Graham et al. 2020). In west-southwest Minas Gerais State of the Brazilian citrus belt, application of the same measures as those in California and Sao Paulo States has ensured an economically sustainable citrus production (Bassanezi et al. 2020). In these scenarios, predictive modeling of CLs and psyllids dispersal allows for the identification of more risky areas, maximizing the efficiency of monitoring and surveillance by paying special attention to specific hotspots (Graham et al. 2020).

**Low/no psyllid infestation and absence of HLB-associated CLs.** In this situation, two basic actions are required: to control insect vector populations and to avoid/eliminate CLs entrance/

sources. Although easy to say, both objectives are very difficult to accomplish. In fact, there are not reported cases of full eradication of *T. erythrae* and *D. citri*, with the only exception of Australia/Northern Territory in Oceania in which the removal of all citrus plants while fighting citrus canker led to *D. citri* extinction (Bellis et al. 2005). It is important to remark that this occurred in a reduced, quite isolated area, in which abroad infections are less probable to occur and limited to certain entry points that can be controlled easily by phytosanitary authorities. Otherwise, the economic, social, and environmental impact of removing all psyllid hosts from a wide area makes this solution unattainable and does not ensure their reentrance by uncontrolled pathways. Psyllid control with *Tamarixia* spp. or other biological agents is effective to reduce vector populations in certain regions favorable for the parasitoid and could be useful in integrated pest management (IPM) programs if infestation level is

**TABLE 2**  
**Representative tools developed worldwide to help fight against Huanglongbing (HLB) spreading**

Web tool	Features	Developer	Website
#cuidemosnuestroscitricos	Web page to inform about HLB disease and prevention strategies in Argentina	Federcitrus-Senasa	<a href="https://federcitrus.org/accionHLB/">https://federcitrus.org/accionHLB/</a>
Citrus Diagnosis App	Symptom-based tool to support the identification of HLB disease	UF/IFAS	<a href="http://www.makecitrusgreatagain.com/">http://www.makecitrusgreatagain.com/</a>
Citrus Diseases	Symptom-based tool to support the identification of HLB disease	USDA-UF	<a href="http://idtools.org/id/citrus/diseases/">http://idtools.org/id/citrus/diseases/</a>
Citrus Greening Solutions	Web page with bioinformatics tools for the host citrus, the vector, and pathogens	USDA-NIFA	<a href="https://citrusgreening.org/">https://citrusgreening.org/</a>
Citrus Pest & Disease Prevention Program	Web page to inform about HLB disease and prevention strategies in California	CDFCA	<a href="https://citrusinsider.org/">https://citrusinsider.org/</a>
Citrus Pests	Web tool to support the identification of pest for adult insects on cultivated citrus	USDA-UF-SPDN	<a href="http://idtools.org/id/citrus/pests/">http://idtools.org/id/citrus/pests/</a>
Doc Citrus	Symptom-based tool to support the identification of citrus disease	Derek Hsieh	<a href="https://appadvice.com/app/doc-citrus/1506258866">https://appadvice.com/app/doc-citrus/1506258866</a>
Drones Imaging	Multispectral sensor to identify HLB symptoms in citrus leaves by drones	Agrowing-Vetorgeo-UNESP	<a href="https://agrowing.com/">https://agrowing.com/</a>
FuturCrop	A software to plan treatments and control psyllid	FuturCrop	<a href="https://futurcrop.com/">https://futurcrop.com/</a>
GIPcitrícos	Web page to inform about citrus pests and diseases and prevention strategies	IVIA	<a href="http://gipcitrícos.ivia.es">http://gipcitrícos.ivia.es</a>
HLB Monitoring Web App	Web app to locate the proximity to confirmed HLB outbreaks	UC ANR	<a href="http://geoportal.ucanr.edu/sandbox/hlb_proximity/">http://geoportal.ucanr.edu/sandbox/hlb_proximity/</a>
Pests and Diseases	Web page to identify and inform about pests and diseases and management	USDA-APHIS	<a href="https://www.aphis.usda.gov/aphis/resources/pests-diseases/pests-and-diseases">https://www.aphis.usda.gov/aphis/resources/pests-diseases/pests-and-diseases</a>
Pests and Diseases of Citrus	Web app to identify citrus plant pests and diseases	MarGroup	<a href="https://play.google.com/store/apps/details?id=com.trigunawan45">https://play.google.com/store/apps/details?id=com.trigunawan45</a>
Plants Diseases Identifier	Symptom-based tool to support the identification of HLB disease	Jose Bello	<a href="https://appadvice.com/app/plants-diseases-identifier/1537766094">https://appadvice.com/app/plants-diseases-identifier/1537766094</a>
Save Our Citrus app	Symptom-based tool to support the identification of HLB disease	USDA-APHIS	<a href="http://saveourcitrus.org/index.php/soc-iphone-app">http://saveourcitrus.org/index.php/soc-iphone-app</a>

low. Besides being eco-friendly, this strategy presents the advantage of reaching backyards and unmanaged areas with alternative hosts, usually not subjected to any chemical treatment (Diniz et al. 2020; Miranda and Ayres 2020). However, as taught by regions with high infestation levels, local treatments are not efficient to solve the problem and IPM must be implemented within an areawide management strategy integrated with PRA tools to improve their outcome. A threshold infestation level should be defined to start insecticide treatments, because both psyllids have high reproductive capacity and the density of their populations grows exponentially once they reach it, thus becoming impossible to control fully (Monzó and Stansly 2015). For example, in Sao Paulo State, the presence of a single psyllid (detected by visual inspection or yellow stick trap) is sufficient to indicate the need for an insecticide spray application (Miranda and Ayres 2020). However, it is important to remark that the best strategy would be to eradicate the psyllids while their populations are still low and restricted to limited regions because, as commonly said, “dead the dog the rabies is gone.” Nevertheless, once the insect vector is established, the appearance of the associated bacterium in the region is usually a matter of time (Table 1).

Early diagnosis and destruction of infected trees are essential if psyllids are already present. Removal of CLs inoculum sources relies on visual identification of infected trees, subsequent confirmation of their infection by molecular techniques and, finally, tree elimination. There is a lag of time (about 4 to 10 months) between tree infection and symptoms development in which these infected presymptomatic plants serve as CLs source, explaining why HLB continues spreading despite removal of symptomatic trees (Gottwald and McCollum 2017; Lee et al. 2015). Confirmation of CLs infection is mostly obtained by qPCR method (Cellier et al. 2020). However, the Ct threshold to consider a sample positive is not standardized worldwide and can lead to underestimate the presence of infected plants. For example, in California, Ct threshold has been recently augmented from 32 to 36.99, although Ct < 38 is widely accepted as indicative of infection (Graham et al. 2020). The irregular distribution of CLs within a plant (Alves et al. 2021; Vasconcelos et al. 2021) constitutes another handicap for diagnosis. To solve this, it is recommended to test a high number of leaves and perhaps different tissues and organs from every candidate tree. Recently, it has been proposed to perform diagnosis using root tissue, where the pathogen is more evenly distributed, reducing the chance of getting negative results from infected samples (Braswell et al. 2020). Current molecular diagnosis methods for CLs are based on bacterial DNA detection by PCR, SSR, PCR-RFLPs, droplet digital PCR, loop mediated amplification technology (LAMP), immune capture-PCR, qPCR, and nested PCR (Hong et al. 2019). Research over the last several years was directed to increase sensitivity and specificity of fast CLs-detection methods. For example, LAMP technology is as sensitive as real-time PCR for symptomatic plants and do not require special equipment, providing a specific, rapid, efficient, and labor-saving methodology that can be applied in the field to confirm CLs infection (Choi et al. 2018). Field-detection kits based on LAMP technology have been developed. Despite advances in all PCR-related diagnosis methods, real-time qPCR techniques, which are specific, precise, sensitive, and relatively fast and economic, are recommended by EPPO and FAO to confirm CLs infection (Cellier et al. 2020). Other detection methods, based on histochemical analysis, thin layer chromatography, monoclonal antibodies, or microscopy are also setup to detect HLB disease (Valdés et al. 2016). Iodine staining to detect starch accumulation, characteristic of infected tissues, can also be used easily in the field (Etxebarria et al. 2007) and do not require high budgets, making it amenable for the less economically sustainable citricultures. Characteristic changes in plant volatile emitted profile are also being used to detect infected trees in the field. Based on CLs-specific induced volatile changes, trained canines can detect infected plants (Gottwald et al. 2020). Besides volatiles, by mass spectrometry imaging analysis and Raman spectroscopy of leaves, increase in certain metabolites/molecules,

associated to HLB infection, can be detected even before symptoms development (de Moraes Pontes et al. 2020; Sanchez et al. 2019). However, all these techniques and tools are more laborious/expensive and/or require more skilled technicians compared with qPCR.

Early development of predictive models for citrus regions, before psyllids/CLs entrance or immediately thereafter, highlighting risk areas, provides a very useful tool to fight HLB. From this point of view, it would be also very useful to encourage citrus growers to coordinate for collectively managing the disease if it is present and, if not, they should organize to set up areawide control before its likely arrival. However, voluntary coordination has showed poor success, and it is suggested to make it mandatory where possible to guarantee an efficient management of psyllids/HLB (Singerman and Useche 2019). Communication campaigns directed to raise awareness about the catastrophic effects that HLB can have on citriculture may increase voluntary involvement of farmers and related stakeholders in areawide management programs. Social awareness can also help to limit illegal import of plant material (especially from infested/infected regions), and it may additionally help identification of HLB symptoms outside commercial orchards.

It is also important that nurseries are prepared before HLB and/or associated insect vectors reach an area. Young flushing materials are preferred hosts for both psyllid species, and preventive protection of the facilities with antiinsect screens must be adopted in citrus nurseries. Although this measure is perceived as uneconomical in no-HLB scenarios, it would effectively reduce the likelihood of the disease to be established and would provide healthy plant material for replantings if it arrives.

## CONCLUSIONS

Prevention is the best way to stop HLB spread, being essential in regions free of dispersing/causal organisms, to prevent their entry through severe quarantine measures and numerous inspections. Source material for grafting and nursery trees intended to planting should be produced under psyllid-proof structures, to ensure they are disease-free. Vectors eradication plans are essential to avoid rapid disease spread in the eventual case any of the HLB-associated bacterium reaches uninfected areas. For an HLB management program to be successful, the elimination of sources of CLs inoculum inside and outside commercial orchards is required, and insecticides should be applied preventatively in order to reduce the primary infection. Area-wide management and PRA tools are essential tools to fight the disease in all scenarios.

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